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(71) Applicant *(for all designated States except US)*: **THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA** [US/US]; Center for Technology Transfer, 3700 Market Street, Suite 300, Philadelphia, PA 19107 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ROOK, Alain, H.** [US/US]; 455 Ballytore Road, Wynnewood, PA 19096 (US). **FRENCH, Lars, E. 7, Leon-Gaud 1206 Geneva** [CH/CH]; - (CH).

(74) Agents: **LICATA, Jane, Massey** et al.; Licata & Tyrrell P.C., 66 E. Main Street, Marlton, NJ 08053 (US).

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(54) Title: COMPOUNDS AND METHODS FOR IDENTIFYING, STAGING AND TREATING CUTANEOUS T-CELL LYMPHOMA

(57) Abstract: A method and composition for treatment of cutaneous T-cell lymphoma is provided which involves administration of recombinant human CD40L. Methods of identifying and staging CTCL are also provided.

COMPOUNDS AND METHODS FOR IDENTIFYING, STAGING AND TREATING CUTANEOUS T-CELL LYMPHOMA

FIELD OF THE INVENTION

This invention relates to treating cutaneous T-cell lymphoma (CTCL) and cancers with recombinant human CD40 ligand (CD40L). This invention also relates to identifying and staging CTCL.

BACKGROUND

Cutaneous T-cell lymphoma (CTCL) is a disease in which T-lymphocyte cells of the lymphatic system become malignant and affect the skin. CTCL localizes to the skin more often than other forms of non-Hodgkin's lymphomas. The etiology of CTCL is unknown. Various theories implicate infectious agents, oncogenes, cytokines or occupational or environmental exposures. While clusters of cases are reported within families, CTCL is considered to be a sporadic disease without any real evidence of transmissibility.

CTCL is a lymphoproliferative disorder typically characterized by infiltration of the skin with clonally derived malignant CD4+ T lymphocytes that phenotypically resemble mature T cells (Diamandidou, E. et al. 1996. *Blood* 88:2385-2409). Early presentation of the disease may be confused with eczema, tinea corpus, or psoriasis. Therapeutic efforts are based on the extent of disease, the integrity of the immune system, and the likelihood for progression of disease. Several additional observations which can affect therapeutic decisions include antitumor immune responses mediated by cytotoxic T cells detected in patients with CTCL; biologic response modifiers, which can augment cytotoxic T-cell responses with other specific arms of the antitumor

response, such as recombinant interferon-α; and the use of combinations of potent chemotherapeutic agents.

The malignant cells in CTCL appear to arise from the expansion of a single clone of T-lymphocytes which bears the phenotype of mature helper T-cells expressing CD4+/CD45RO+. During advancing stages of CTCL a decline in cell-mediated immunity is typically observed, characterized by depressed cell-mediated cytotoxicity and deficient responsiveness of T-cells to antigens and mitogens. Studies of the nature of the malignant T-cells have provided evidence that these cells are at least partially responsible for the generation of the immune defects by way of production of T-immunosuppressive T-helper type 2 (Th2) cytokines and by the depressed production of T-helper type 1 (Th1) cytokines. A marked defect in interleukin-12 (IL-12) production is also observed in CTCL at all stages, but most profoundly in advanced stages of CTCL. This defect may underlie the profound deficiency in anti-tumor immunity. Notably, IL-12 therapy of CTCL can induce lesion regression and anti-tumor cytotoxic T-cell responses.

For patients with limited or early stage disease, the cell-mediated immune response is usually normal, and the likelihood for serious systemic disease progression is low. Accordingly, these patients can be treated effectively with a variety of skin-based therapies, including topical mechlorethamine, topical carmustine, and psoralen and ultraviolet A light (PUVA). A significant percentage of patients with such early disease appear to be cured of their disease with these treatments.

Advanced forms of CTCL are not as easily cured, and are often fatal. The patient with advanced forms of CTCL may present with malignancies that progress from plaques to tumors. A more common form of advanced CTCL, Sezary Syndrome, involves erythroderma occurring throughout the course of disease. In Sezary Syndrome, the malignant cell population, which has an early propensity to localize within the upper

dermis, and particularly, within the epidermis (epidermotropism), also becomes nonepidermotropic and is associated with deeper dermal extension and involvement of the peripheral blood. Concurrent with this leukemic, progressive phase of the disease, is the onset of progressive immunologic dysfunction. Among the constellation of immune abnormalities that have been noted are increased serum IgE, decreased T cell responses to antigens, impaired cellular cytotoxicity, and peripheral eosinophilia. Associated with these immune abnormalities is a striking deficiency in the ability of peripheral blood mononuclear cells (PBMC) to produce interferon-γ and interleukin-2 in response to activation signals (Rook, A.H. et al. 1993. *Arch. Dermatol.* 129:486; Vowels, B.R. et al. 1992. *J. Invest. Dermatol.* 99:90). In contrast to a defect in production of T-helper type 1 (Th1) cytokines, upon stimulation, PBMC from patients with Sezary Syndrome produce increased concentrations of interleukin-4, the levels of which correlate with numbers of circulating malignant T cells (Vowels, B.R. et al. 1992. *J. Invest. Dermatol.* 99:90).

Recent studies have shown that excess interleukin-4 production by PBMCs from Sezary Syndrome patients can be inhibited *in vitro* either by interferon-γ or by interferon-α (Vowels, B.R. et al. 1992. *J. Invest. Dermatol.* 99:90). Moreover, Sezary Syndrome patients who develop complete remission associated with the disappearance of detectable malignant peripheral blood cells during therapy with biologic response modifiers, including interferon-α, have restored a normal pattern of cytokine production by their PBMC in concert with the normalization of many immune parameters (Vowels, B.R. et al. 1993. *J. Invest. Dermatol.* 100:556). Therefore, strategies directed simultaneously at affecting the cytokine imbalance and impeding proliferation of the malignant T-cell population may have a beneficial effect on the outcome of this frequently fatal disorder.

Interleukin-12 (IL-12) is a cytokine that is a powerful inducer of interferon-γ production and that exerts potent Th1-inducing effects during the evolution of immunologic responses against certain microbial antigens (Chan, S.H. et al. 1991. *J. Exp. Med.* 173:869-879; Hsieh, C.S. et al. 1993. *Science* 260:547-549). IL-12 augments Natural Killer (NK) cell cytotoxicity and cytotoxic T cell proliferation and function (Hiramatsu, K. et al. 1998. *Cancer Immunol. Immunother.* 46:1-6; Haku, T. et al. 1997. *Cytokine* 9:846-852; Sahin, U. et al. 1996. *Cancer Immunol. Immunother.* 42:9-1), activities that may be beneficial in regard to the abnormal Th2 clonal proliferation observed in advanced CTCL, including Sezary Syndrome. Studies have shown that PBMCs isolated from patients with advanced CTCL exhibit marked defects in the production of IL-12 (Rook, A.H. et al. 1997. *Clin. Exp. Immunol.* 107:16-20; Seo, N. et al. 1998. *Clin. Exp. Immunol.* 112:403-409). Further, IL-12 has been shown to have potent antitumor activity in mice with transplantable and primary tumors (Nishimura et al. 1995. *Immunol. Lett.* 48:149-152) and in mice with metastatic residual lymphoma (Verbik et al. 1996. *Clin. Exp. Metastasis* 14:219-229). Brunda and colleagues (1993. *J. Exp. Med.* 178:1223) have demonstrated antitumor activity of IL-12 in mice following both systemic and intralesional administration.

Recent *in vitro* experiments have also shown that the depressed interferon-γ production observed in peripheral blood mononuclear cells isolated from patients with advanced CTCL is normalized by the addition of recombinant IL-12. These *in vitro* studies also showed that the depressed cell-mediated cytotoxicity in CTCL is augmented by IL-12 (Rook, A.H. et al. 1996. *Ann. NY Acad. Sci.* 795:310-318; Rook et al. 1995. *J. Immunol.* 154:1491-1498).

The present invention provides methods of identifying, staging and treating CTCL.

SUMMARY OF THE INVENTION

An object of the present invention is to provide a method of treating CTCL in humans comprising administering to a human an effective amount of recombinant human CD40L in an amount which effectively enhances the TH1-mediated immune responses of a patient.

Another object of the present invention is to provide a method of staging cancer comprising identifying a human patient as having cancer, determining the level of expression of CD40L in the patient and comparing said level with the expression level of CD40L previously expressed by the patient or with an expression level of CD40L found in a healthy control.

DETAILED DESCRIPTION OF THE INVENTION

Recent evidence has been provided showing that *in vivo* activation of CD40 in mice (using agonistic anti-CD40 antibodies) replaces T-cell help that is required for priming of effector T-cells, and moreover, augments anti-tumor vaccine efficacy by reverting peripheral tolerance. It has now been found that T-lymphocytes from patients with a severe form of CTCL, namely Sezary Syndrome, have a defect in production of CD40L in response to T-cell activation signals. This defect in CD40L expression results in defective production of TH1 cytokines, Interferon gamma (IFNγ), IL-12 and tumor necrosis factor alpha (TNFα) by lymphocytes and/or antigen presenting cells. Using a novel recombinant form of human CD40L (ApoTech Research & Development, Epalinges, Switzerland) IL-12 and TNFα production was restored by antigen presenting cells of CTCL patients. Therefore, recombinant human CD40L is useful for the immunotherapy of CTCL and other types of cancers, including but not limited to melanoma, lymphoma, leukemia, breast, colon cancer, lung cancer and such other cancers that benefit from enhanced TH1-mediated immune responses.

The present invention provides a useful method for alleviating symptoms and treating CTCL in patients which comprises administering a recombinant soluble human form of CD40L to the patient. The method of the present invention is particularly useful in treating advanced Cutaneous T-cell Lymphoma, which includes patients presenting with plaques, Sezary Syndrome, or tumors with large cell transformation. Recombinant soluble human form of CD40L can be administered either systemically (*e.g.*, subcutaneously, intravenously) or intralesionally and can be formulated in any pharmaceutically acceptable carrier which is known to those of skill in the art. An effective dose of a recombinant soluble human form of CD40L is administered repeatedly, for a period of time. Generally, treatment would be extended for a period of months or until symptoms of CTCL (such as plaques, tumors or erythroderma) regress or are reduced. An effective dose is one in which there is at least a partial response in the patient. A partial response is considered to be a decrease in physical symptoms, a reverse in defective TNF alpha production, or a reverse in defective IL-12 production in patients with CTCL. In some patients a reduction of symptoms of advanced CTCL represents a total absence of lesions after treatment. Dosage schedules and regimens are routinely designed by those of skill based upon results described below.

The recombinant soluble human form of CD40L may be administered either alone or in combination with therapeutic agents or adjunct therapies that are targeted to CTCL treatment. Examples of adjunct therapeutic agents and adjunct therapies include, but are not limited to, interferon alpha, interferon gamma, vitamin A derivatives (Acitretin, Bexarotene), photopheresis and UVA phototherapy.

Also provided by this invention is a method of staging cancer in a human by identifying the human patient as having cancer and then evaluating the immune responsiveness of the patient by comparing the level of expression of CD40L. CD40L

expression is decreased in Sezary Syndrome patients as compared to healthy controls and also decreases as the severity of the disease increases (i.e. as the tumor burden is increased). The expression of CD40L in a patient directly correlates with the overall immune responsiveness of the patient. As described in Example 1, Sezary Syndrome patients with high circulating tumor burden have a more pronounced defect in CD40L expression than those patients with a low tumor burden. Accordingly the present invention provides a method of staging cancer comprising identifying a human patient as having cancer, determining the level of expression of CD40L in the patient and comparing said level with the expression level of CD40L previously expressed by the patient or with an expression level of CD40L found in a healthy control.

The following nonlimiting examples are provided to further describe the invention.

EXAMPLES

Example 1

Defective expression of CD40L on T-cells of patients with CTCL (Sezary Syndrome) following T-cell activation showed normal CD40 expression on antigen presenting cells of patients with CTCL.

FACS analysis of CD40L expression on CD4+ T-lymphocytes in response to T-cell activation by anti-CD3 showed that CD40L surface expression is decreased in these patients as compared to healthy controls. Further, patients with high circulating tumor burden were found to have a more pronounced defect in CD40L expression than those patients with low tumor burden.

FACS analysis of CD40L expression on activated (anti-CD3) or non-activated (vehicle) CD4+T cells of 5 normal donors and 7 Sezary Syndrome patients showed decreased induction of CD40L upon activation in Sezary Syndrome patients. The CD40L induction rate for normal donors was found to be approximately

twice as great as the induction rate for Sezary Syndrome patients (not inclusive of Sezary Syndrome patients with low tumor burden). FACS analysis of CD40 expression on CD14+/CD64- antigen presenting cells (APC) of a healthy control and high tumor burden Sezary patient showed conserved expression of CD40 in both cases.

Example 2

Defective IL-12 production by peripheral blood mononuclear cells (PBMC) of Sezary patients in response to T-cell activation by anti-CD3 was measured. The levels of IL-12 were measured by ELISA in the culture media of PBMC from five normal donors and Sezary Syndrome patients following anti-CD3 stimulation for 48 hours, and found that the IL-12 production in normal donors was at least approximately 15 times greater in normal donors as compared with Sezary Syndrome patients. The levels of IL-12 measured by ELISA in the culture media of PBMC from a normal donor and 3 Sezary Syndrome patients in response to LPS and interferon gamma (IFNγ), illustrated that direct stimulation of Sezary Syndrome patients APC's are able to produce IL-12 in response to direct (LPS/IFNγ) as opposed to indirect T-cell mediated (anti-CD3) stimulation.

Example 3

Defective TNF alpha production by PBMC of Sezary patients in response to T-cell activation by anti-CD3 was measured. The levels of TNF measured by ELISA in the culture media of PBMC from five normal donors and five Sezary Syndrome patients following anti-CD3 stimulation for 48 hours were measured, with the TNF production being at least twice as great in Sezary Syndrome patients not exhibiting a low tumor burden. Similarly, the levels of TNF measured by ELISA in the culture media of PBMC from a normal donor and 3 Sezary Syndrome patients in response to LPS and IFNγ were measured. The results illustrated that direct stimulation to Sezary Syndrome

patients' APC's produced TNF in response to direct (LPS/IFNγ) as opposed to indirect T-cell mediated (anti-CD3) stimulation.

Example 4

Defective IFN (interferon gamma) production by PBMC of Sezary patients in response to T-cell activation by anti-CD3 was measured. Levels of IFN measured by ELIZA in the culture media of PBMC from five normal donors and five Sezary Syndrome patients following anti-CD3 stimulation for 48 hours revealed an approximate twenty-five times lower production rate of IFN in the Sezary Syndrome patients not exhibiting low tumor burden as opposed to the normal donors.

Example 5

The effect of soluble recombinant human CD40L on the expression of costimulatory molecules (CD83, CD86) and IL-12 by purified human dendritic cells (DC) derived from CD14+ monocytes was studied. Recombinant human CD40L were found to strongly upregulate both CD83 and CD86 expression on dendritic cells. Dendritic cells were exposed to media alone, LPS (20mg/ml:positive control), CD40L (5μg/ml) for 24 hours and CD83 and CD86 expression was assessed by FACS. Further, when dendritic cells were exposed to media alone, LPS (20ng(ml: positive control), CD40L (5μg/ml) or another TNF family member LIGHT (5μg/ml)for 24 hours and measured for IL-12 expression assessed by ELISA, the recombinant human CD40L was also found to induce IL-12 production by dendritic cells.

Example 6

Induction of IL-12 production by interferon gamma matured PBMC of healthy donors upon stimulation with recombinant human CD40L. In this study, the levels of IL-12 were measured by ELISA in the culture media of PBMC from a normal donor when exposed to IFNγ(1000U/ml) + mouse recombinant human CD40L. Levels of IL-12 were also measured by ELISA in a culture

media of PBMC from a normal donor (ND) when exposed to IFNγ(1000U/ml) + mouse recombinant CD40L (muCD40L 10 μg/ml plated in wells);IFNγ(1000U/ml) + human recombinant CD40L (huCD40L 10 μg/ml plated in wells) or IFNγ alone (1000U/ml: negative control). The level of IL-12 in the IFN/ human recombinant CD40L sample was found to be approximately at least 2.5 times as great as the IFN/mouse recombinant CD40L sample; and 5 times as great as the IFN only sample.

Example 7

Induction of IL-12 production by interferon gamma matured PBMC of Sezary patients by exposure to soluble recombinant human CD40L was evaluated. Levels of IL-12 were measured by ELISA in the culture media of PBMC from a normal donor (healthy control) and 3 different Sezary Syndrome patients following exposure for 48 hours to the following agents: PBS (US), IFN(1000U/ml), IFN(1000U/ml)+LPS (1 μg/ml), CD40L (1 μg/ml), and IFN(1000U/ml)+CD40L (1 μg/ml). The recombinant human CD40L corrected the ability of the Sezary Syndrome patients to generate an anti-tumor response. The Sezary Syndrome patient with the lowest tumor burden exhibited elevated IFN-LPS; CD40L; and IFN-CD40L levels as compared to the Sezary Syndrome patient with moderate tumor burden. Both the Sezary Syndrome patient with low tumor burden and the Sezary Syndrome patient with moderate tumor burden exhibited elevated CD40L and IFN-CD40L levels as compared to the Sezary Syndrome patient with high tumor burden.

Example 8

Induction of TNF alpha production by interferon treated PBMC of Sezary patients by exposure to soluble recombinant human CD40L was studied. Levels of TNF alpha were measured by ELISA in the culture media of PBMC from a normal donor (Healthy control) and 3 different Sezary Syndrome patients following exposure for 48 hours to the following agents: PBS

(US), IFN(1000U/ml), IFN(1000U/ml)+LPS (1 μg/ml), CD40L (1 μg/ml) and IFN(1000U/ml)+CD40L (1 μg/ml). The Sezary Syndrome patient with the lowest tumor burden exhibited elevated IFN-LPS; CD40L; and IFN-CD40L levels as compared to the Sezary Syndrome patient with moderate tumor burden. Both the Sezary Syndrome patient with low tumor burden and the Sezary Syndrome patient with moderate tumor burden exhibited elevated CD40L and IFN-CD40L levels as compared to the Sezary Syndrome patient with high tumor burden.

What is Claimed is:

1. A method of treating cancer in a human comprising administering to a human an effective amount of recombinant human CD40L.

2. The method of claim 1 further comprising administering an adjunct therapeutic agent.

3. A composition for treatment of cutaneous T-cell lymphoma in a human comprising recombinant human CD40L and an adjunct therapeutic agent.

4. A method of staging cancer comprising identifying a human patient as having cancer, determining the level of expression of CD40L in the patient and comparing said level with the expression level of CD40L previously expressed by the patient or with an expression level of CD40L found in a healthy control.